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Preparation of highly effective antibacterial coating with polydopamine/chitosan/silver nanoparticles via simple immersion

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ABSTRACT

In this study, we report a simple and facile method to fabricate an antibacterial composite coating by combining polydopamine (PDA), chitosan (CS) and silver nanoparticles (AgNPs) on urinary catheter and titanium (Ti) surfaces. The preparation consists of a co-deposition of PDA and CS in acid solution and subsequently immersion into silver nitrate ($AgNO_3$) solution. This approach allows the antibacterial PDA/CS/AgNPs coating to be prepared for several hours by the simple immersion process under friendly conditions, and the surface morphology for coated catheters illustrated that CS plays a key role in improvement of the coating smoothness. The antibacterial tests against *S. aureus* were performed on the measurements of the inhibition halo and adhered bacterial numbers. The results showed that the PDA/CS/AgNPs-coated catheter has long-lasting stability for more than 30 days and antibacterial activity against *S. aureus*, and the antiadhesion rates of PDA/CS/AgNPs-coated Ti surface are 91 % and 85 % for live and dead bacteria, respectively. The PDA/CS/AgNPs coating based on the simple immersion has great potential in practical application for antibacterial urinary catheters and other biomedical devices.

1. Introduction

Urinary catheter is used on an intermittent or indwelling basis for the patient in order to relieve urinary retention and incontinence. However, the complications occur during long-term using the urinary catheter in the clinical setting, and the most common of problems is the development of catheter-associated urinary tract infection (CAUTI) [1–3]. The incidence of CAUTI stems from the fact that the urinary catheter provides ideal conditions for the bacteria colonization and the development of enormous biofilm populations. Many bacterial species colonize indwelling catheters as the biofilms, which induces complications in patients' care [3].

Nowadays, many studies focus on the coating using the surface-modifying macromolecules additives to solve the practical issues, and the modified surfaces exhibit better hydrophilicity, antifouling and other advantages [4–7]. For the issue of bacterial biofilm, the central factor in pathogenesis of CAUTI, altering the surface modified by macromolecules additives to inhibit biofilm formation is a good method for preventing infection. As the risk of infection increases with the catheterization time in the human body, it is ideal to develop a long-lasting and highly efficient antibacterial coating for catheters with excellent biocompatible and cost-effective properties. Currently several types of antibacterial macromolecules are available for reducing bacterial colonization [8]. As a biomacromolecule, Chitosan (CS) has been extensively studied to be potential antibacterial material for biomedical devices due to its non-toxicity, biocompatibility and antimicrobial properties [9–19]. CS-coated surfaces can significantly reduce adhesion for many kinds of bacteria, including *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans* etc [10]. To further improve antibacterial efficiency, the antibacterial composite of CS and silver nanoparticles (AgNPs) were prepared since AgNPs have the broad-spectrum antimicrobial activity [20]. This composite exhibited more effective antibacterial activities and low cytotoxicity [17,10–19].

To prepare a long-lasting antibacterial CS/AgNPs coating, there is a critical need to develop a method to reinforce the CS/AgNPs coating. Inspired by the adhesive proteins secreted by mussels for strong attachment to inorganic and organic surfaces, polydopamine (PDA) as the surface anchor has been an interesting coating material since its first reported application in 2007 [21]. PDA is durable and biocompatible, and can be used to readily prepare the functional coating materials via grafting methods or post-modification functionalization [22–24]. It has

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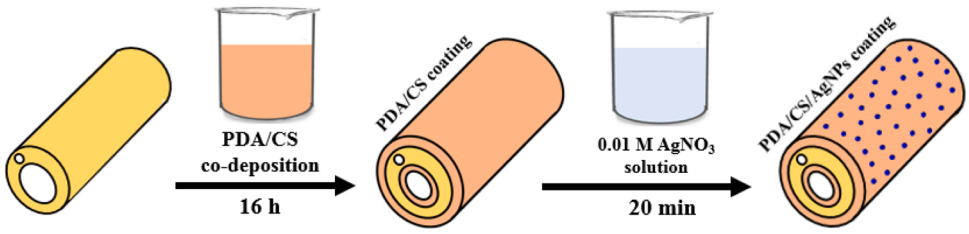

Fig. 1. Schematic diagram of procedures for preparation of PDA/CS/AgNPs coating on catheter segment.

been shown that the PDA coating as an intermediate bridging layer can offer active sites to couple with CS [25,26]. Therefore, PDA/CS/AgNPS composite coating is expected to have long-lasting and improved antibacterial efficiency. To our knowledge, there was only one study focusing on a composite coating composed of PDA, hydroxyapatite (HA), AgNPs, and CS on titanium (Ti) substrates by a multilayer assembly process for antimicrobial and osteogenesis. The coating procedures included several steps, and was very time-consuming, which took for a few days [19].

In this study, we report a simple immersion method to prepare the PDA/CS/AgNPs composite coating on the urinary catheter and Ti surfaces, which only takes for a few hours. Here, Ti surfaces is adopted as the second substrate since it is a widely used metallic biomaterial. The procedure mainly consists of a co-deposition of PDA and CS in acid solution since CS is only soluble in acid solution, which successfully forms the PDA/CS coating. To our knowledge, no co-deposition of PDA and CS in acid solution has been reported. In the above PDA coating preparation, the dopamine polymerization involves the oxidation of catechol in dopamine to quinone by alkaline pH-induced oxidation [21]. However, the dopamine polymerization must be induced by adding an oxidant in neutral or acidic aqueous media [27], thus ammonium persulfate (AP) was chosen as the oxidant in this work. Subsequently, the PDA/CS-coated samples were immersed into 0.01 M silver nitrate solution, in which monodispersed AgNPs were formed and absorbed by the hydroxyl groups [23,28] and the PDA/CS/AgNPs composite coating was prepared. Finally, *in vitro studies* were performed to determine the stability and the antibacterial performance of the prepared PDA/CS/AgNPs coating. There are some studies on antibacterial characteristic of the polymer [29–31] and stainless steel [23] surfaces modified with the PDA/AgNPs coating. The PDA/AgNPs coating was also prepared on the urinary catheter to test the antimicrobial difference between the PDA/AgNPs and PDA/CS/AgNPs coatings in this work, which can be used to illustrate the effect of CS on preventing bacteria adhesion for the PDA/CS/AgNPs coating.

2. Experimental section

2.1. Materials

Dopamine hydrochloride (98 %, Mw =600 Da), CS (Mw = 50,000–190,000 Da) AgNO3 (99 %) and AP (99 %) were purchased from Sigma-Aldrich (U.K.). All reagents were of analytical grade and used without further purification. Hydrochloric acid (HCl: 99 %) was chosen as acidic aqueous media and the acid solution (pH = 3.8) was adjusted by adding deionized water. Latex urinary catheters with silicon treatment (STAR 14Fr 5−10 ml) were purchased from China. Since the urinary catheter is curved surface, it is difficult to measure the contact angle values. Thus, Ti metal plate (thickness 2 mm, size 3 cm × 3 cm) was also used as substrate. The absolute ethanol (Sigma-Aldrich, Gillingham, U.K.) was used to clean the samples.

2.2. Preparation of PDA/CS/AgNPs coating

The urinary catheter was cut into 2 cm long segments and then cleaned ultrasonically with absolute ethanol and deionized water, respectively prior to use. Preparation process of the PDA/CS/AgNPs coating on the catheter segments was presented in Fig. 1. CS, dopamine hydrochloride and AP with the same concentration of 0.1 g/L was successively dissolved in HCl solution (pH = 3.8) for stirring 30 min at 60 °C. The colour of the DA solution had a gradual change from colourless to dark brown. The urinary catheters were immersed in the above solution for 16 h at 60 °C and the PDA/CS coating with a thickness of around 130 nm formed on the both inside and outside surfaces of the catheter segments. It was noted that no precipitates were observed after 16 h immersion (photographs not shown), which is important for preparing uniform coating. Subsequently, the PDA/CS-coated samples were rinsed with deionized water and dried under the room temperature (25 °C), and then immersed in 0.01 M $AgNO_3$ solution for 20 min at 60 °C. Finally, the resulting samples were rinsed with ethanol and deionized water and then dried under the room temperature. Ti substrates were also coated by the same process.

2.3. Surface characterization

A scanning electron microscope (field emission-scanning electron microscope (FE-SEM), JEOL JSM-7400 F, Tokyo, Japan) was employed to study the morphology of the coated surface with the accelerating voltage of 5 kV. For the surface composition analysis, energy-dispersive X-ray spectrometry (EDX, QX200, Bruker Ltd., Billerica, U.S.A.) was used with the accelerating voltage of 15 kV. Contact angle was measured by a sessile drop method with a Dataphysics OCA-20 contact angle analyzer (DataPhysics Instruments GmbH, Filderstadt, Germany), and the average contact angle was obtained by measuring six different positions on one sample. All measurements were performed at room temperature.

2.4. Antibacterial tests

To study the long-term antibacterial property of the PDA/CS/AgNPs coating, the PDA/CS/AgNPs-coated catheters segments were immersed in phosphate-buffered saline (PBS) and maintained at a room temperature for 30 days, and then taken out to perform inhibition halo test toward *S. aureus* F1557. Furthermore, the anti-adhesion ability of the PDA/CS/AgNPs-coated catheters against *S. aureus* F1557 was qualitatively assessed by using SEM. Here, culture of *S. aureus* was grown to the mid exponential phase (10^6 CFU/mL). The uncoated and coated catheter segments were immersed vertically in 3 mL of the prepared bacterial suspension (10^6 CFU/mL) and incubated at 37 °C for 3 days (the culture medium was refreshed every day). The catheter segments were taken out, rinsed gently with sterile deionized water to remove loosely adhered bacteria and then dried at room temperature. Subsequently, bacterial adhesion on the outer surface of catheters were observed by using SEM.

In addition, the antibacterial efficacy of PDA/CS/AgNPs-coated Ti substrate against *S. aureus* was assessed by using fluorescence microscopy. The uncoated and coated Ti substrates were immersed vertically in the prepared bacterial suspension at 37 °C for 3 h. The samples were rinsed gently with sterile deionized water and followed by staining with a LIVE/DEAD Baclight bacterial viability kit L13152 (Fisher Scientific, Loughborough, U.K.). The fluorescence microscope (OLYMPUS BX 41,

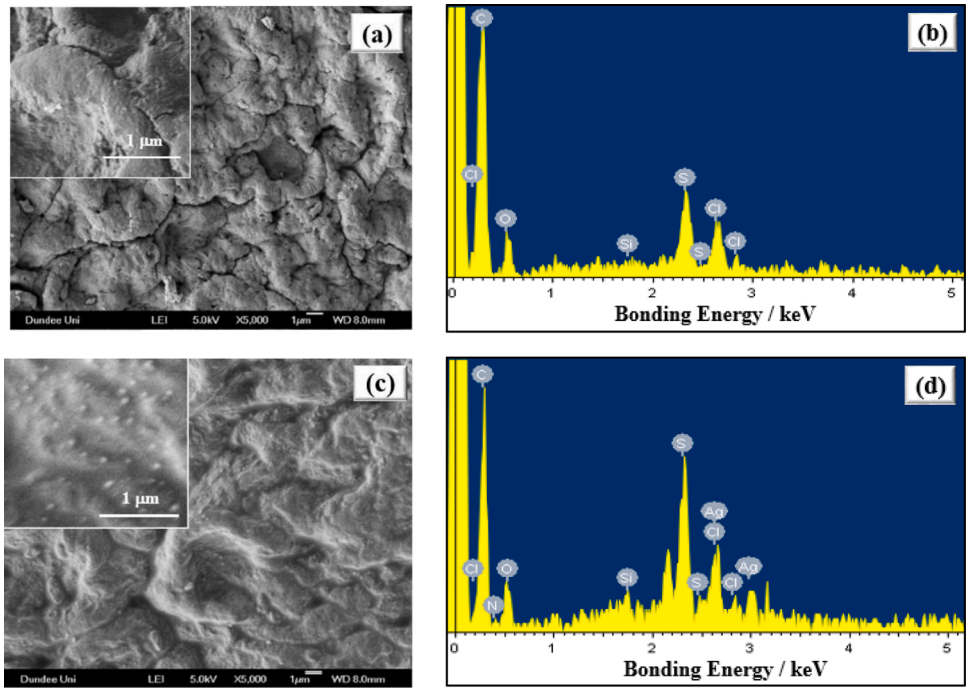

Fig. 2. SEM images of (a) uncoated and (c) PDA/CS/AgNPs-coated catheter surface; EDX results of (b) uncoated and (d) PDA/CS/AgNPs-coated catheter surface.

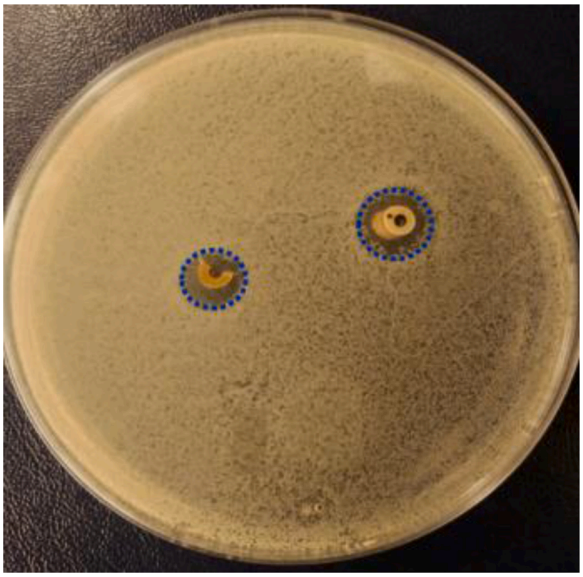

Fig. 3. Inhibition halo tests against *S. aureus* for PDA/CS/AgNPs-coated catheters after immersed into PBS solution for 30 days and then incubated in agar plate for 24 h.

Tokyo, Japan) was used to observe the adhered bacterial cells and adhered bacterial numbers were quantified by using Image Pro Plus software (Media Cybernetics, Rockville, U.S.A.).

3. Results and discussion

3.1. PDA/CS/AgNPs-coated catheter

Surface morphology of the uncoated and PDA/CS/AgNPs-coated catheters was characterized by using SEM and presented in Fig. 2. The uncoated catheter with silicon treatment (Fig. 2a) presents a rough morphology and there are cracks on the surface. The surface constituents of the uncoated catheter include C, O, S, Si, and Cl as shown in Fig. 2b. Compared with the uncoated surface, the PDA/CS/AgNPs-coated catheter surface (Fig. 2c) presents the better morphology and the cracks disappear. Further magnified SEM image as presented in subpicture shows that nanoparticles with an average diameter around 40 nm uniformly distribute on the surface, while the nanoparticles are not observed for the PDA/CS-coated catheter surface (photographs not shown). The surface constituents of the PDA/CS/AgNPs-coated catheter include C, O, S, Si, Cl, N and Ag as presented in Fig. 2d, which verifies the distribution of Ag nanoparticles on the coating. In preparation of PDA/CS/AgNPs-coated catheter, it was found that the temperature, the immersion time and the concentrations of CS, dopamine hydrochloride and AP affect the thickness and morphology of the coating as well as the size of AgNPs. The reason may be their effect on the speed and degree of PDA polymerization and immobilization of CS and PDA. The effect of those parameters on the morphology and surface chemical composition of the coating will be systematically analyzed for the optimization of PDA/CS/AgNPs coating in future works.

The durability and antibacterial activity of PDA/CS/AgNPs coated catheters were evaluated by examining their inhibition halo tests against *S. aureus*. The PDA/CS/AgNPs-coated catheters immersed into PBS solution for 10, 20, and 30 days, respectively, and then incubated in agar plate for 24 h. The result of the inhibition halo test for 30 days is presented in Fig. 3. The clear inhibition zones are observed around the half and the whole coated catheter segments, verifying that the PDA/CS/AgNPs-coated catheter (inside and outside surfaces) have long-lasting stability for more than 30 days and antibacterial activity against *S. aureus*.

Anti-adhesion efficacy of the PDA/CS/AgNPs-coated catheter was examined through the culturing in *S. aureus* suspension for 3 days and then was observed by using SEM. The bacterial adhesion on the uncoated and PDA/CS/AgNPs-coated catheter are presented in Fig. 4. As illustrated in Fig. 4a, lots of bacteria (in red circles) adhered to the surface and the bacteria preferred to attach to the valley and cracks of the uncoated catheter surface. However, only two bacteria adhered in the viewing zone for the PDA/CS/AgNPs-coated surface as shown in Fig. 4b. The results indicate that the PDA/CS/AgNPs-coated catheter surface exhibits remarkable improvement in preventing bacteria

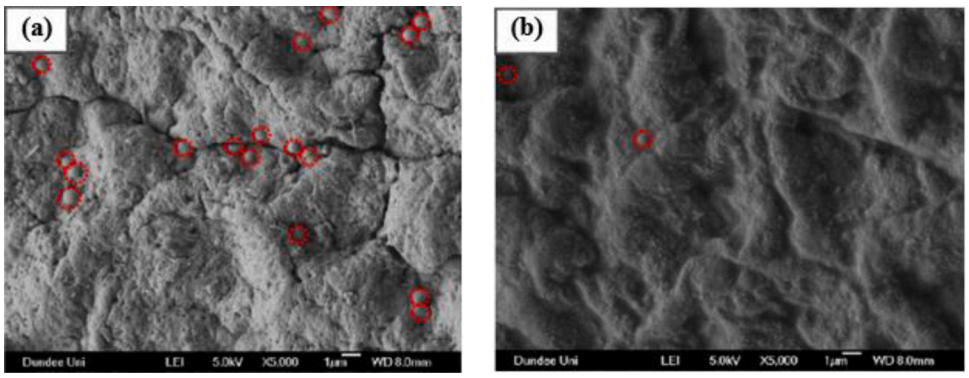

Fig. 4. SEM images of bacterial adhesion for (a) uncoated (b) PDA/CS/AgNPs-coated catheter surfaces after immersed into *S. aureus* suspensions (PBS solution, 10^6 CFU/mL) at 37 °C for 3 days.

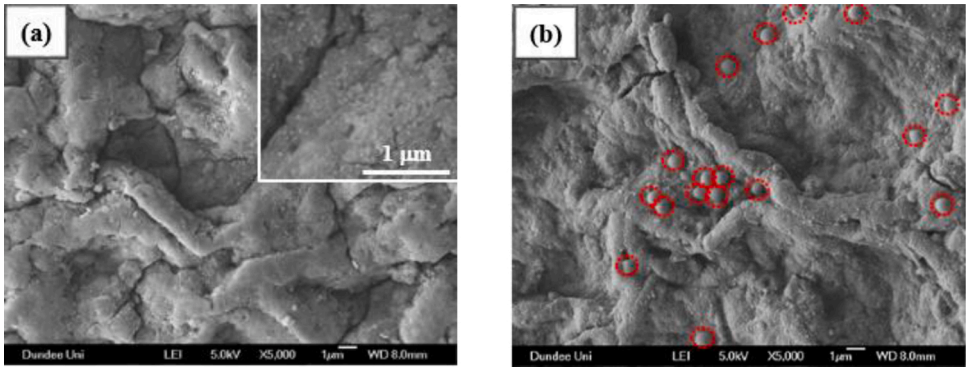

Fig. 5. SEM images of (a) surface morphology and (b) bacterial adhesion for PDA/AgNPs-coated catheters.

Fig. 6. Contact angle of a deionized water droplet on (a) uncoated Ti substrate, (b) PDA/CS-coated and (c) PDA/CS/AgNPs-coated Ti substrate.

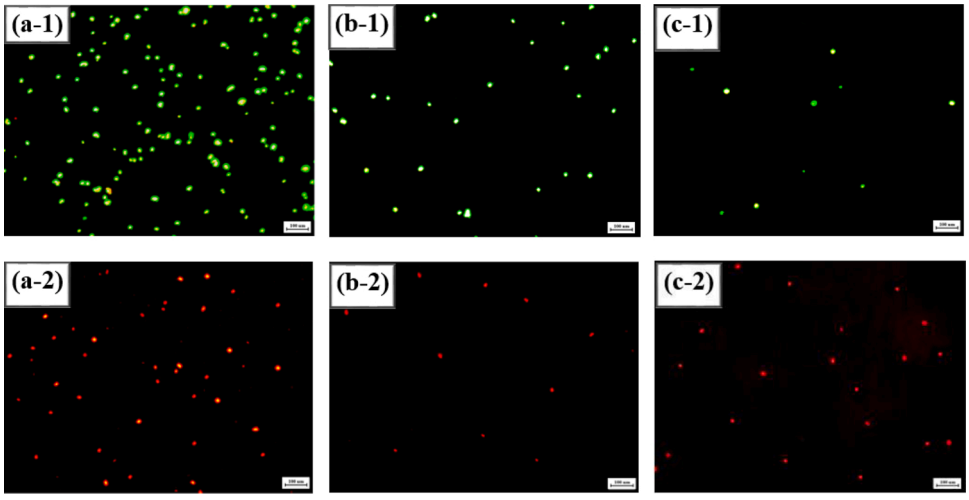

Fig. 7. Live/dead fluorescent images of adhered bacteria on (a) uncoated, (b) PDA/CS coated and (c) PDA/CS/AgNPs coated Ti surfaces after incubated in *S. aureus* suspension for 3 h.

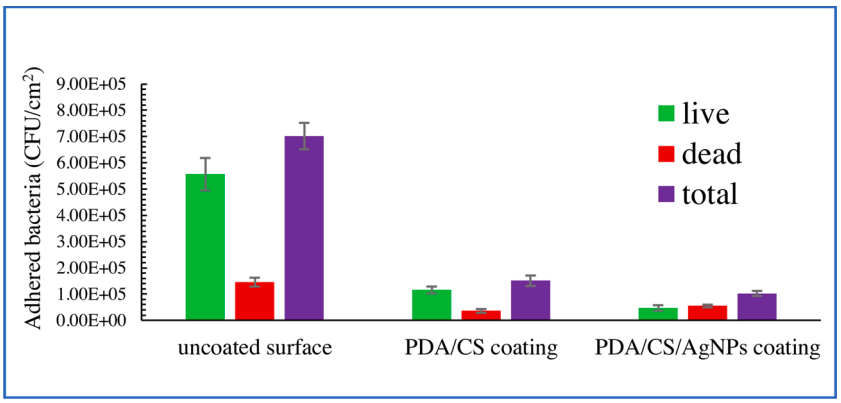

Fig. 8. Comparison of adhered bacteria on Ti surface, PDA/CS coated and PDA/CS/AgNPs coated Ti surfaces after incubated in *S. aureus* suspension for 3 h. (bars represent standard deviation of the mean).

adhesion.

To illustrate effect of CS on preventing bacteria adhesion, SEM images of the surface morphology and the bacterial adhesion for PDA/AgNPs-coated catheter are presented in Fig. 5. The PDA/AgNPs-coated catheter was prepared by the same preparation procedure, the difference is that no CS was added in the acid solutions. As illustrated in Fig. 5a, the PDA/AgNPs-coated surface presents the rough morphology. Compared with the morphology of PDA/CS/AgNPs coating as shown in Fig. 2c, the results indicate that CS improves the coating smoothness. In addition, the size of Ag nanoparticles for the PDA/AgNPs coating is smaller as presented in the subpicture. The reason is speculated that CS molecules have abundant hydroxyl groups and more Ag can be absorbed on the PDA/CS/AgNPs coating. Furthermore, more hydroxyl groups can control the Ag ions release rate, as a result, the PDA/CS/AgNPs coating has long-lasting antibacterial activity (more than 30 days) as shown in Fig. 3. In addition, compared to the bacterial adhesion on the uncoated catheter surface as shown in Figs. 4a, there is better bacterial anti-adhesion for the PDA/CS/AgNPs coating than the PDA/AgNPs coating. These results illustrate that the CS in the PDA/CS/AgNPs coating plays a key role in optimization of the coating morphology and prevention of the bacterial adhesion.

3.2. PDA/CS/AgNPs-coated Ti surface

PDA/CS-coated and PDA/CS/AgNPs coatings were also successfully prepared on the Ti smooth substrate. Contact angles of a deionized water droplet for the uncoated, the PDA/CS-coated and PDA/CS/AgNPs-coated surfaces are shown in Fig. 6. The Contact angles for uncoated Ti surface, PDA/CS-coated surface and PDA/CS/AgNPs-coated surface are $39.2 \pm 1.6°$, $30.8 \pm 1.1°$ and $46.1 \pm 1.2°$, respectively. The results indicated that the PDA/CS/AgNPs coating is less hydrophilic than PDA/CS coating due to the formation of AgNPs on the PDA/CS coating.

The initial adhesion of *S. aureus* on the uncoated, PDA/CS-coated and PDA/CS/AgNPs-coated Ti substrates was evaluated by the fluorescence microscopy. Live/dead fluorescent images and quantitative counts of adhered bacteria are presented Figs. 7 and 8, respectively. Compared to the uncoated Ti substrate (Fig. 7a-1 and a-2), the PDA/CS-coated surface remarkably reduces the live/dead bacterial adhesion as shown in Fig. 7 (b-1) and (b-2), and the antiadhesion rates of the live and dead bacteria are 79 % and 75 % (Fig. 8), respectively. For the PDA/CS/AgNPs-coated surface as shown in Fig. 7(c-2), the dead bacteria on the PDA/CS/AgNPs coating slightly increases when compared to the PDA/CS-coated surface. It is speculated that some live bacteria have been killed by AgNPs, as a result, the number of live adhered bacteria decreases as shown in Figure 7(c-1). The total number of live and dead bacteria on the PDA/CS/AgNPs coating are much less than on the PDA/CS coating and the anti-adhesion rates for live and dead bacteria further increase to 91 % and 85 % (Fig. 8), respectively. These results show that PDA/CS/AgNPs coating has high-efficiency to inhibit the biofilm formation by combining the anti-adhesion property of CS and anti-bacterial property of AgNPs.

4. Conclusion

In summary, a two-step coating method has been developed to preparing PDA/CS/AgNPs antibacterial coatings in acid solution by combining PDA strong attachment, CS anti-adhesion and AgNPs antimicrobial activity. Only simple immersion of the catheter or Ti substrate into the reagent containing solutions is required to prepare the uniform PDA/CS/AgNPs coatings. The thickness of the coating and the size of AgNPs can be controlled by the immersion time and other parameters, such as pH value, temperature and concentration of PDA, CS and AP. CS in the coating plays a key role in optimization of the coating morphology and prevention of the bacterial adhesion. *in vitro studies* showed that the PDA/CS/AgNPs coating has long-lasting antibacterial (>30 days) and high antiadhesion performance for live bacteria (91 %), the reason is speculated that abundant hydroxyl groups in CS molecules can reinforce AgNPs and control the Ag ions release. The positive results obtained in this study is that the PDA/CS/AgNPS composite coating via the simple immersion is expected to be a promising candidate for the development of long-lasting and high-efficiency antibacterial coatings for urinary catheters and other biomedical devices. To further optimize the antibacterial ability, the influence factors and the formation mechanism of PDA/CS/AgNPs composite coating on different substrates will be analyzed in future works. Besides, the release rate of Ag ions and the cell cytotoxicity of the coating will be performed for its practical applications.

Data availability statement

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

CRediT authorship contribution statement

Bing-Bing Wang: Methodology, Investigation, Writing - original draft. **Yu-Hua Quan:** Data curation, Investigation. **Zhi-Ming Xu:** Writing - review & editing. **Qi Zhao:** Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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